PB86-158573

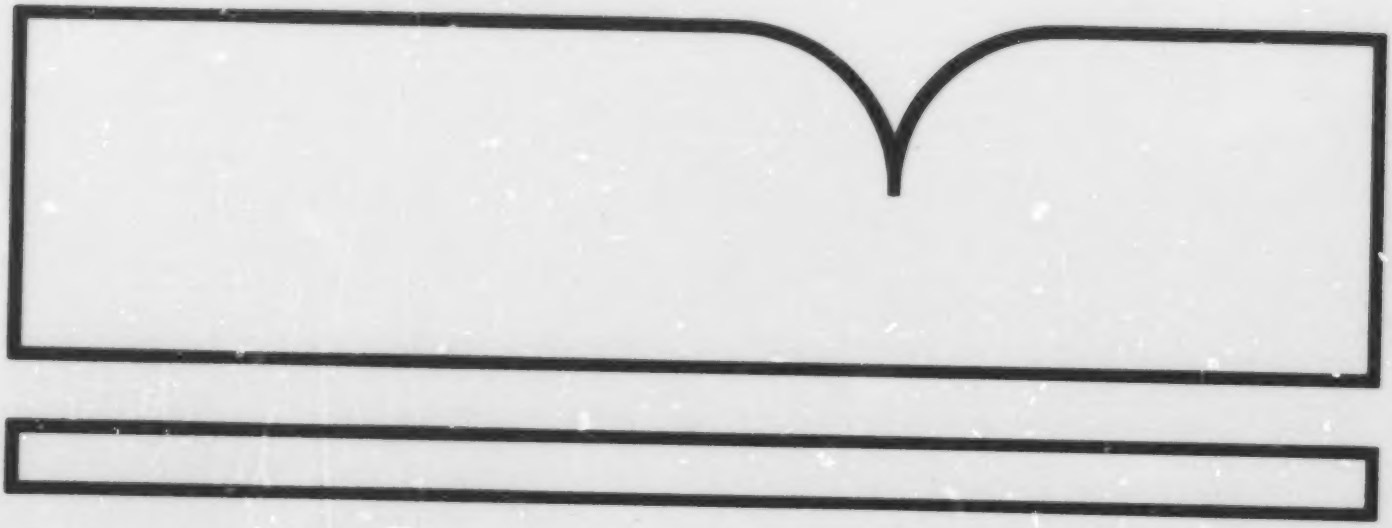
Induction of DNA-Protein Crosslinks in Human Cells by Ultraviolet and Visible Radiations: Action Spectrum

Argonne National Lab., IL

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1985



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/J-85/264	2.
3. PB86-158573	
4. TITLE AND SUBTITLE: Induction of DNA-protein crosslinks in human cells by ultraviolet and visible radiations: action spectrum (Journal Version)	5. REPORT DATE: 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): J.G. Peak, M.J. Peak, R.S. Sikorski, and C.A. Jones	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Arbonne National Laboratory, Argonne, IL	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.: IAG DW89930217
12. SPONSORING AGENCY NAME AND ADDRESS: EPA/HERL/GTD/CMB, MD-68 RTP, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE: EPA/600/11

15. SUPPLEMENTARY NOTES: Photochemistry & Photobiology, Vol. 41, No. 3, pp 295-302, 1985. This paper accepted in lieu of Final Report.

16. ABSTRACT

Abstract—DNA-protein crosslinking was induced in cultured human P3 teratocarcinoma cells by irradiation with monochromatic radiation with wavelengths in the range 254–434 nm (far-UV, near-UV, and blue light). Wavelength 545 nm green light did not induce these crosslinks, using the method of alkaline elution of the DNA from membrane filters. The action spectrum for the formation of DNA-protein crosslinks revealed two maxima, one in the far-UV spectrum that closely coincided with the relative spectrum of DNA at 254 and 290 nm, and one in the visible light spectrum at 405 nm, which has no counterpart in the DNA spectrum. The primary events for the formation of DNA-protein crosslinks by such long-wavelength radiation probably involve photosensitizers. This dual mechanism for DNA-protein crosslink formation is in strong contrast to the single mechanism for pyrimidine dimer formation in DNA, which apparently has no component in the visible light spectrum.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field Group

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED	21. NO. OF PAGES: 9
	20. SECURITY CLASS (This page): UNCLASSIFIED	22. PRICE: $ 9.95

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

EPA/600/J-85/264
JOURNAL ARTICLE

Photochemistry and Photobiology Vol. 41, No. 3, pp. 295 – 302, 1985
Printed in Great Britain. All rights reserved

Copyright © 1985 Pergamon Press Ltd

INDUCTION OF DNA–PROTEIN CROSSLINKS IN HUMAN CELLS BY ULTRAVIOLET AND VISIBLE RADIATIONS: ACTION SPECTRUM

J. G. Peak,[1] M. J. Peak,[1] R. S. Sikorski[1] and C. A. Jones[2]

[1]Photobiology Group and [2]Carcinogenesis Group, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL 60439, USA

(*Received* 25 *July* 1984; *accepted* 12 *November* 1984)

Abstract—DNA–protein crosslinking was induced in cultured human P3 teratocarcinoma cells by irradiation with monochromatic radiation with wavelengths in the range 254–434 nm (far-UV, near-UV, and blue light). Wavelength 545 nm green light did not induce these crosslinks, using the method of alkaline elution of the DNA from membrane filters. The action spectrum for the formation of DNA–protein crosslinks revealed two maxima, one in the far-UV spectrum that closely coincided with the relative spectrum of DNA at 254 and 290 nm, and one in the visible light spectrum at 405 nm, which has no counterpart in the DNA spectrum. The primary events for the formation of DNA–protein crosslinks by such long-wavelength radiation probably involve photosensitizers. This dual mechanism for DNA–protein crosslink formation is in strong contrast to the single mechanism for pyrimidine dimer formation in DNA, which apparently has no component in the visible light spectrum.

INTRODUCTION

The biological significance of covalent crosslinking between DNA and protein is not yet clearly understood. It has been observed that such crosslinks induced in the cells of xeroderma pigmentosum patients after exposure to 254 nm UV radiation are more persistent than those induced in cells of normal patients, suggesting that the enhanced susceptibility of these patients to skin cancer of solar etiology may in part result from this lesion (Fornace and Kohn, 1976).

Covalent bonds between DNA and protein can be formed under a variety of circumstances. For example, such crosslinks can be induced in cells by exposing them to carcinogenic or procarcinogenic chemicals, such as alkylating agents (Thomas, 1975; Shooter and Wiessler, 1976; Schulein *et al.*, 1978; Magana-Schwenke and Ekert, 1978; Olinski *et al.*, 1981), mycotoxins (Moule *et al.*, 1982; Ireland *et al.*, 1982; Fornace and Little, 1979), or amino derivatives (Fornace and Little, 1979; Rayshell *et al.*, 1983; Murray and Meyn, 1983). Crosslinks have also been induced by irradiation of cells with X-rays (Yamamoto, 1975), broadband near-UV radiation (Bradley *et al.*, 1979; Anderson *et al.*, 1976), and monochromatic radiation [at wavelengths from 254 nm (Fornace and Kohn, 1976; Magana-Schwenke and Ekert, 1978; Olinski *et al.*, 1981; Okada *et al.*, 1978; Schimmel *et al.*, 1976; Smith, 1975; Todd and Han, 1975; Eisenstark *et al.*, 1982), 365 nm, and 405 nm (Han *et al.*, 1983)].

Powerful sources are now available for many wavelengths of monochromatic UV and visible light, enabling us to study many biological effects of this band of the electromagnetic spectrum and correlate these effects with wavelength. The energies of photons produced by these sources are well matched with chemical bonding energies in biological molecules. Examples of studies with monochromatic sources include correlations between radiation-induced pyrimidine dimer formation and DNA breakage, on the one hand, and mutagenic and lethal events on the other hand (Rothman and Setlow, 1979; Rosenstein and Setlow, 1980; Kantor *et al.*, 1980; Peak and Peak, 1983; Rosenstein and Ducore, 1983). It has recently become evident that longer wavelengths (> 320 nm) of radiation exert their mutagenic and lethal effects upon cells by as yet unknown mechanisms radically different from those of shorter (< 320 nm), more energetic wavelengths (Webb, 1977; Peak and Peak, 1982; Jagger, 1981). There is good evidence that far-UV (wavelengths < 320 nm) radiation is directly absorbed by DNA, causing the formation of photoproducts, such as pyrimidine dimers of the cyclobutane type, adducts of the 6-4 bipyrimidine type (Varghese and Wang, 1967; Brash and Haseltine, 1982), or pyrimidine–purine adducts (Bose *et al.*, 1982). In contrast, near-UV (wavelengths > 320 nm) radiation has been clearly shown to damage DNA by indirect mechanisms, involving nonDNA photosensitizers and reactive states of oxygen; these mechanisms have been discussed in reviews (Webb, 1977; Peak and Peak, 1982; Jagger, 1981). The specific lethal and mutagenic changes induced by these wavelengths are still under study.

Little is known about the mechanism(s) of the effects of the longer wavelengths of near-UV radiation and visible light on mammalian cells. The near-UV and visible radiations from the sun are known to penetrate Caucasian skin at least to the dermis and to irradiate significant numbers of cells in the peripheral circulation (Parrish, 1983).

Pyrimidine dimer formation plays a progressively smaller role in the formation of DNA lesions as wavelength increases (Peak and Peak, 1982); these radiations may kill mammalian cells *via* lesions in DNA other than pyrimidine dimers (Smith and Paterson, 1982; Suzuki *et al.*, 1981; Zelle *et al.*, 1980). For example, after 405 nm irradiation, only DNA breaks and DNA–protein crosslinks have been measured (Rosenstein and Ducore, 1983; Han *et al.*, 1983), but some evidence suggests that DNA breaks may not actually be lethal to the irradiated cells (Rosenstein and Setlow, 1980; Elkind and Han, 1978).

This study is an investigation of the spectral dependence of the formation of DNA–protein crosslinks in the UV and visible regions. Crosslinks may play a significant role in the lethal and mutagenic consequences to cells exposed to nonionizing radiation. This study has been made technically feasible by the recent development of highly sensitive filter alkaline elution methods (Kohn *et al.*, 1974; Kohn *et al.*, 1981), which permit detection of the DNA fraction that binds to filters unless treated with an enzyme such as proteinase K.

MATERIALS AND METHODS

Cells and culture conditions. Human teratocarcinoma cells (designated P3) were a gift from Dr. E. Huberman (Huberman *et al.*, 1984). These epithelial cells were seeded at 2×10^6 cells/100-mm-diameter Petri plate in RPMI-1640 medium (Gibco) supplemented with 20% fetal calf serum, 2 m*M* glutamine, 100 IU/mℓ penicillin, and 100 μg/mℓ streptomycin. [^{3}H]-Thymidine was added at 0.05 μCi/mℓ (20 Ci/mmol) to the cultures for 48 h to radiolabel the cellular DNA; longer or shorter labeling periods did not affect subsequent results. Immediately prior to harvesting, the cells were washed with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline* and incubated for 1 h in unlabelled medium. The cells were then dissociated in trypsin/EDTA (Huberman *et al.*, 1984), washed once with PBS-A, and resuspended in 2×10^6 cells/mℓ in PBS-A prior to irradiation. This suspension was maintained at 0°C–0.5°C until lysis of the cells. This period never exceeded 2 h, and no significant loss in cell viability was observed in control, unirradiated cells as a result of this treatment. Cell viability was determined by cloning efficiency. Stained colonies were counted after growth in Petri dishes for 7–8 days.

Irradiation procedures. Radiation at wavelengths of 290, 313, 334, 365, 405, 434, and 545 nm was obtained from a 2500 W high-pressure Hg–Xe lamp in a Kratos-Schoeffel housing coupled to a 500 mm Bausch and Lomb grating monochromator. Radiation at 254 nm was obtained from a low-pressure Hg^* Penray lamp. The fluence rates (W m^{-2}, Peak and Peak, 1982), filters (Webb and Peak, 1981), and Morowitz correction factors (Morowitz, 1950) [to eliminate the effects of cell shielding (self absorption) at shorter wavelengths] used in the experiments are shown in Table 1. Fluence rates at 254 nm were measured with a calibrated GE germicidal meter, model UV 480, and at all other wavelengths were measured with a calibrated YSI-Kettering radiometer. Cells were irradiated in a gently stirred suspension, in jacketed square quartz (below 320 nm) or Pyrex (above 320 nm) vessels.

Assays for DNA–protein crosslinks. Alkaline elution methods as originally described by Kohn *et al.* (1974), modified as described below were used to assay for DNA–protein crosslinks. Cells (5×10^5) in PBS-A were pipetted onto cooled polyvinyl chloride filters (Millipore, 2 μm BSWP02500, previously saturated with PBS-A) and washed four times with cold PBS-A. The cells were lysed by dripping 5 mℓ of ice-cold lysis solution (2 *M* NaCl, 0.04 *M* EDTA, 0.2 *M* Sarkosyl, pH 10.0) through the filters. The lysates were then washed with 10 mℓ of 0.02 *M* EDTA, pH 10.0. To determine the effect of proteinase K upon elution profiles, we washed the lysates with TEN-SDS buffer (0.01 *M* Tris, 0.01 *M* EDTA, 0.01 *M* NaCl, 0.5% sodium dodecyl sulfate) containing 0.5 mg/mℓ of proteinase K (Sigma) for 30 min, after which the buffer and proteinase K were allowed to pass through the filter. (The proteinase K was tested before use to ensure that there was no contamination by DNAse.) Alkaline elution was performed with 30 mℓ of 0.1 *M* tetrapropyl-ammonium hydroxide in 0.02 *M* EDTA, pH 12. The elution rate was 0.035 mℓ/min, and 10 fractions were collected at 90 min intervals. Since it was not possible to obtain linear dose responses for all wavelengths using this method, presumably because the radiations induced both DNA–protein crosslinks and backbone breaks, we also used the method of Kohn *et al.* (1981) exactly as described. A portion of each UV

Table 1. Fluence rates, filters, and Morowitz correction factors for the wavelengths used

Wavelength (nm)	Fluence rate (W m^{-2})	Filters	Morowitz factor†
254	1	Ultraviolet Products 6-275	0.45
290	26	Corning 0-56	0.65
313	260	Corning 0-53	0.83
334	160	Corning 7-51	0.88
365	710	Corning 0.52*	0.91
405	400	Optics Technology LP400	0.92
434	600	LP400	0.93
545	400	LP400	0.95

*Ground to half thickness. †Scattered light determined for 2×10^6 cells /mℓ from spectral scans kindly determined by Dr D. A. Haugen, using a Beckman spectrophotometer UV 5260, with the cuvette set at the position for maximal scatter.

*Abbreviations: ethylene diaminotetraacetic acid, EDTA; phosphate-buffered saline, PBS-A; tris-EDTA-NaCl-sodium dodecyl sulfate, TEN-SDS.

irradiated sample was exposed to 900 rads from a ^{60}Co γ-ray source (1 min exposure) before the cells were applied to the polyvinyl chloride filters. Alkaline elution was performed after cell lysis with or without the proteinase K treatment. Using data from elution profiles with and without γ-ray treatment we calculated dose responses for the induction of apparent DNA–protein crosslinks using the formula of Kohn *et al.* (1981). To avoid room light-induced DNA breakage, we performed the elutions under dim yellow light.

RESULTS

Alkaline elution profiles of DNA from these cells with and without proteinase K treatments (no γ-ray treatment) after various fluences of 290 nm, 313 nm, 365 nm UV and 434 nm blue light are shown in Fig. 1, and the results of similar experiments after 545 nm green light exposure in Fig. 2. In all control experiments (cells not treated with UV radiation), the bulk of the DNA (> 90%) remained on the filters throughout the elution. After all exposures of these cells to UV and blue light (254 to 434 nm), but not after exposure to green 545 nm light, two effects upon the subsequent elution profiles were observed. The rate of elution increased for cells without

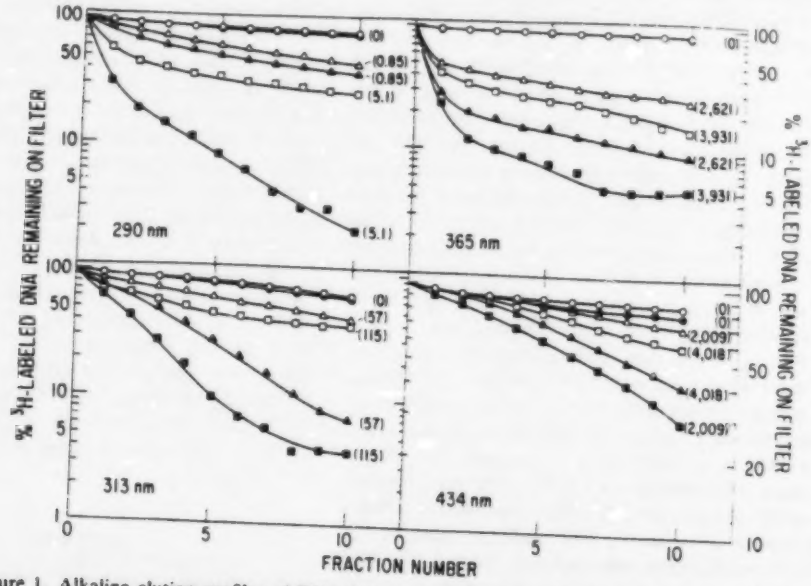


Figure 1. Alkaline elution profiles of DNA from irradiated human P3 teratocarcinoma cells. Circles represent unirradiated controls; triangles and squares represent samples irradiated at the various fluence levels given in parenthesis (kJ m^{-2}). Open symbols are for samples not treated with proteinase K; solid symbols are for proteinase K-treated samples. In the case of the unirradiated controls the data points with or without proteinase K treatments often superimpose.

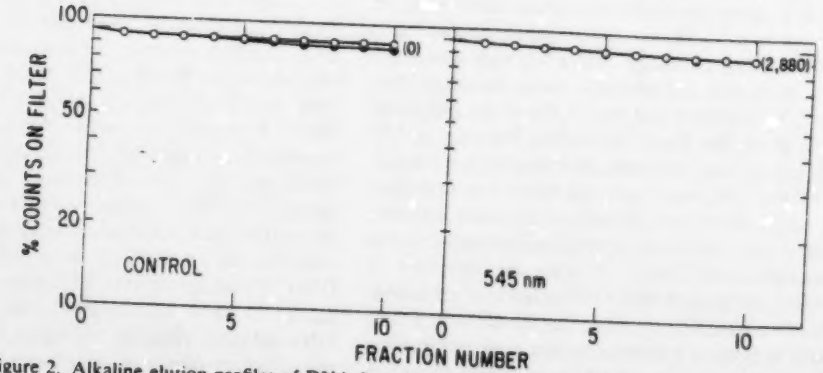


Figure 2. Alkaline elution profiles of DNA from human P3 teratocarcinoma cells irradiated with and without 2.88×10^6 J m^{-2} 545 nm green light, and with and without proteinase K treatments. In the case of the unirradiated controls the data points with or without proteinase K treatments often superimpose. The fluences used are shown in parentheses. Open symbols correspond to no proteinase K treatment; filled symbols, to proteinase K treatment.

proteinase K treatments, reflecting the induction of DNA breaks by these wavelengths of UV light. It has previously been shown that UV of wavelengths as long as 405 nm can induce alkaline-labile lesions in DNA and that about 80% of these are frank single-strand breaks (Peak and Peak, 1982). Figure 1 also shows that proteinase K treatments of DNA from cells exposed to these UV radiations further enhances the rates of elution from the filters whereas proteinase K has no effect on the rate of elution of DNA from unirradiated cells. This is evidence that, after exposure of the cells to these radiations, a fraction of the DNA that is retained on the filters after both SDS and pH 12 treatments elutes following treatment with proteinase K. Because it is unlikely that bonds weaker than covalent bonds would survive both the detergent and the high pH treatments, this elution pattern suggests that irradiation with wavelengths between 254 nm and 434 nm induces the attachment of protein adducts to the DNA by covalent bonds. This conclusion is based on the assumption that Kohn's interpretations of alkali elution profiles are correct. Treatment of DNA from cells irradiated with 545 nm green light showed no DNA breaks or proteinase K dependent increase in the fraction of DNA eluting from the filters (Fig. 2).

The types of results described by Fig. 1 are refractory to satisfactory analysis of fluence responses for the induction of DNA–protein crosslinks, partially because two DNA lesions that affect the elution profiles—DNA breakage and DNA–protein crosslinking—are being measured simultaneously. Kohn *et al.* (1981) have described a method to obtain a value for the apparent numbers of DNA–protein crosslinks induced, by first breaking the DNA into fast-eluting small fragments using X or γ rays. The increase in retention following UV treatment then gives a measure of the cross-linking effect. Figure 3 shows the elution profiles under such conditions using wavelengths of radiation at 254, 313, 365, and 405 nm. As shown previously, treatment with UV alone caused DNA to elute faster from the filter than unirradiated DNA (open circles) as a result of DNA breakage. After 900 rads γ rays (no UV) there was considerably more breakage than after UV treatment and only 2–3% of the DNA was retained on the filter. Increasing fluences of UV radiation caused increasing fractions of the γ irradiated DNA to be retained on the filters (closed circles) as a result of crosslink formation. (In control experiments it was shown that this increase in retention was reversed to the levels of retention obtained in controls not treated with UV radiation in treatment with proteinase K, indicating that the DNA had been linked to protein.) Similar results were obtained at 290 and 434 nm, but not at 545 nm, where there was no increase in the binding of DNA to the filters with increased fluence. The relative efficiencies for the induction of these apparent DNA–protein crosslinks caused by 254–434 nm radiation were calculated, using the formula of Kohn (1981) assuming 2.43 DNA breaks per 10^8 dalton induced by 900 rads of gamma radiation as quoted by Rosenstein and Ducore (1983). The fluence response curves are shown in Fig. 4. At all these wavelengths, DNA–protein crosslinks were induced linearly and reproducibly over the fluence ranges used in these experiments.

Figure 5 is an action spectrum for the induction of DNA–protein crosslinks in human cells by radiation from 254 nm to green light at 545 nm, a wavelength at which no DNA–protein crosslinks induction could be detected. The data show that at 254 and 290 nm, the relative efficiency per quantum for the induction of DNA–protein crosslinks conforms to the DNA spectrum of Setlow (1974), however insufficient data are available to exclude totally the possibility that the crosslink spectrum may conform to protein. Above these wavelengths there is increasing deviation from the fit to DNA. For wavelengths above 330 nm, more DNA–protein crosslinks are induced in the DNA than is warranted by the direct absorption of photons by DNA. We have also described a similar excess of pyrimidine dimers (Peak *et al.*, 1984b) and pyrimidine adducts (unpublished observations) induced in DNA by 334 nm. Figure 5 also shows that there is a secondary peak for the induction of DNA–protein crosslinks at 405 nm, about three orders of magnitude less efficient than the far UV peak at 254 nm. Radiation at 365 and 434 nm induced DNA–protein crosslinks at about the same efficiency, about half an order of magnitude less than at 405 nm.

DISCUSSION

We have described here for the first time an action spectrum for the induction of DNA–protein crosslinks in cells. At the two shortest wavelengths studied, this spectrum apparently coincides with the spectrum of DNA, suggesting that apparent DNA–protein crosslinks may be formed by these photons by a mechanism involving direct absorption of photons by DNA. However, fewer coinciding points were measured than observed for the induction of dimers (Rothman and Setlow, 1979; Smith and Paterson, 1982) and DNA breakage (Rosenstein and Ducore, 1983) in mammalian cells. At the investigated wavelengths longer than 290 nm, deviation from the DNA spectrum for induction of apparent DNA–protein crosslinks (not necessarily the same as those induced by far UV radiation) suggests the participation of as-yet-unidentified chromophores other than DNA. Consistent with our understanding of the actions of these wavelengths is the possibility that DNA–protein crosslinks are induced via photodynamic effects involving reactive states of oxygen generated from excited photosensitizers (Peak *et al.*, 1983; Peak *et al.*, 1984a). Candidates for photosensitizers include porphyrin and its derivatives, which have absorption maxima near 405 nm (Pruckner and Stern, 1936), close to the near-UV peak at 405 nm for

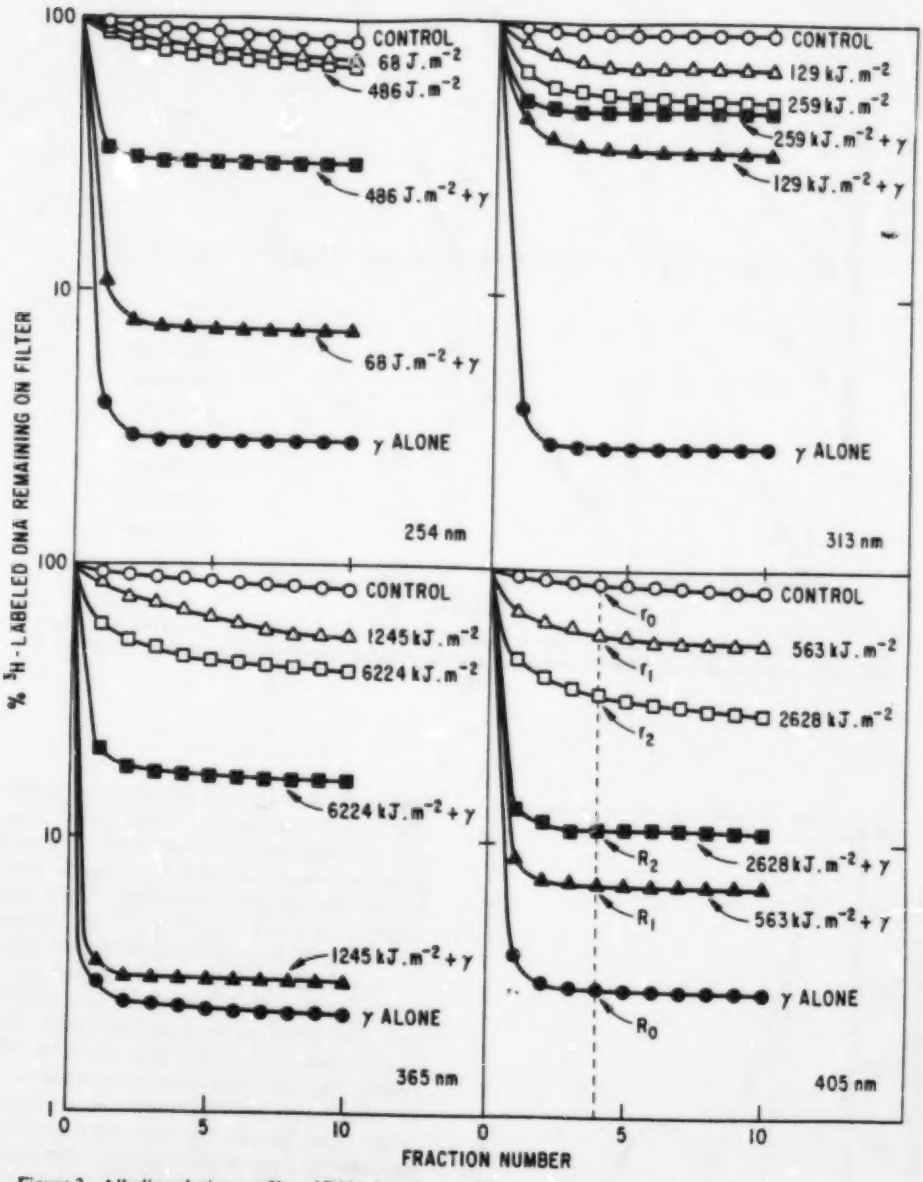


Figure 3. Alkaline elution profiles of DNA from human P3 teratocarcinoma cells irradiated with different wavelengths of UV radiation, with and without 900 rads of ^{60}Co γ-ray exposure. Circles are for controls with no UV radiation exposure; triangles and squares are for different fluences of UV radiation; open symbols, no γ-ray irradiation; filled symbols, with 900 rads γ-ray irradiation. R_0, R_1, R_2, r_0, r_1 and r_2 are values used in the calculation of relative apparent DNA–protein crosslinks as described by Kohn (1981).

DNA–protein crosslinks. For DNA breaks in human fibroblasts, an absorption maximum near 460 nm was found, evidence for bilirubin as the sensitizer in that effect (Rosenstein and Ducore, 1983). From this finding, it is logical to conclude that many nonDNA chromophores may participate as photosensitizers of induced damage to DNA caused by near-UV radiation (Peak *et al.*, 1984a).

The biological significance of DNA–protein crosslinks is not clear. If it is assumed (a) that the sites of DNA–protein crosslinks are uniformly distributed along the genome, (b) that each fragment of γ-ray-exposed DNA retained on the filter (Fig. 3) has an average of one site of DNA–protein crosslinking, and (c) that the mitochondrial DNA content of the cells (< 1%) is insignificant compared with the nuclear DNA, then it becomes possible to estimate the number of crosslinking sites induced in the

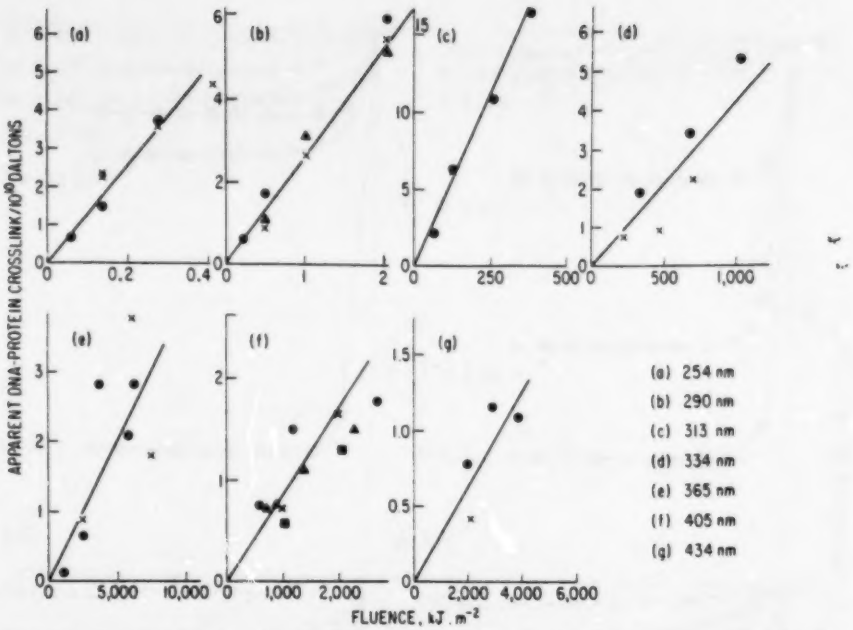


Figure 4. Dose responses for the induction of apparent DNA–protein crosslinks by seven wavelengths of UV and visible radiations. Different symbols represent replicate experiments performed on different days.

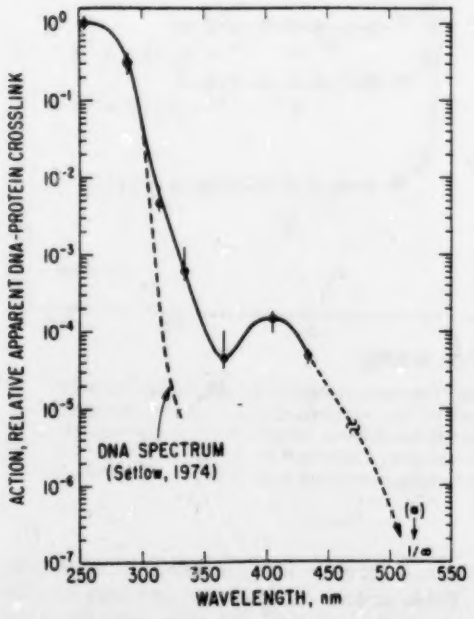


Figure 5. Action spectrum for the relative induction of apparent DNA–protein crosslinks by UV and visible radiations. The units for relative action were corrected for quantum efficiency. Error bars represent one standard deviation of the curve. For comparison, the spectrum of DNA (Setlow, 1974) has been superimposed, using a normalized scale.

genome per lethal event by the different wavelengths (Table 2). Values for F_{37} (fluence for 37% survival) were obtained for colony formation after irradiation under the same conditions as for our measurements here of the induction of DNA–protein crosslinks (Jones *et al.*, 1983). In Table 2, we compare the number of DNA–protein crosslinks and dimers per F_{37}, nonlinearly related to one lethal event, after irradiation by each of these wavelengths (254–434 nm). The dimer values were recalculated from the data of Han *et al.* (1983) and Smith and Paterson (1982), the only values available for dimer induction in mammalian cells by radiation at wavelengths longer than 320 nm. The reason for the differences between these two sets of values for dimer induction is not known. Below 320 nm, we find approximately 1 DNA–protein crosslink per 2×10^4 dimers or about 40 DNA–protein crosslinks per lethal event. Since the cells appear to survive more than 2×10^3 DNA–protein crosslinks induced at longer wavelengths (405 nm), it would appear that the small fraction of DNA–protein crosslinks induced by the shorter wavelength radiations are not important in UV lethality to cells, providing that no interactions between dimers and DNA–protein crosslinks are occurring. At longer wavelengths, the reciprocal argument would seem to apply. As cells survive more than 10^4 dimers induced by short wavelength radiation, it seems unlikely that the small number of dimers induced at 405 nm, for instance, plays a role in

Table 2. Relative induction of DNA–protein crosslinks (dpc) and pyrimidine dimers in mammalian cell DNA by UV and visible radiations

Wavelength (nm)	F_{37}* (human)	dpc/genome/F_{37} (human)	dimers/genome/F_{37} (human)**	dimers/genome/F_{37} (CHO)†
254	14	48	727080	182000
290	31	26		
313	3650	58	953568 ‡	
334	510000	946		
365	1710000	206	61020	26500
405	3590000	992		4360
434	4200000°	>450		

*J m $^{-2}$. **Smith and Paterson. †Han *et al.* ‡310 nm. °F_{37} not determined.

cell killing. It remains an open question as to whether excess DNA–protein crosslinks in cells irradiated with 365, 405, and 434 nm are the lesions that kill the cells.

Apparently cells can survive relatively large numbers of these insults, if the assumptions underlying, Table 2, are correct, before dying, perhaps as many as one DNA–protein crosslinks per five genes. If it is assumed that DNA–protein crosslinks could block gene functions, then this suggests that these lesions can be rapidly repaired by the cell.

Acknowledgements—This investigation was supported in part by PHS Grants 85456 and 85461 awarded by the National Cancer Institute, DHHS, in part by the U.S. Department of Energy under contract No. W-31-109-ENG-38, and in part by the Environmental Protection Agency under interagency agreement DW 930217-01-0. We thank Dr. D. A. Haugen for performing the spectral measurements necessary to calculate the Morowitz correction factors, and Dr. E. M. Westbrook for his contribution to the manuscript.

REFERENCES

Anderson, E., Y. Nakashima and W. Konigsberg (1976) *Nucleic Acids Res.* **2**, 361–371.
Bose, S. N., R. J. H. Davies, S. K. Sethi and J. A. McCloskey (1982) *Science* **220**, 173–175.
Bradley, M. D., I. C. Hsu and C. C. Harris (1979) *Nature* **282**, 318–320.
Brash, D. E. and W. A. Haseltine (1982) *Nature* **289**, 189–192.
Eisenstark, A., S. Kovaks and J. Terry (1982) *J. Natl. Cancer Inst.* **69**, 177–181.
Elkind, M. M. and A. Han (1978) *Photochem. Photobiol.* **27**, 717–724.
Fornace, A. J. and K. W. Kohn (1976) *Biochim. Biophys. Acta* **453**, 95–103.
Fornace, A. J. and J. B. Little (1979) *Cancer Res.* **39**, 704–710.
Han, A., M. J. Peak and J. G. Peak (1984) *Photochem. Photobiol.* **39**, 343–348.
Huberman, E., C. K. McKeown, C. A. Jones, D. R. Hoffman and S-I. Murao (1984) *Mutat. Res.* **130**, 127–137
Jagger, J. (1981) *Photochem. Photobiol.* **34**, 169–268.
Jones, C. A., M. J. Peak and E. Huberman (1983) Abstracts of the 14th Annual Meeting of the Environmental Mutagen Society, March 3–6, San Antonio, Texas, p. 110.
Kantor, G. J., J. C. Sutherland and R. B. Setlow (1980) *Photochem. Photobiol.* **31**, 459–464.
Kohn, K. W., R. A. G. Ewig, L. G. Erickson and L. A. Zwellig (1981) In *DNA Repair 1. A Laboratory Manual of Research Procedures* (Edited by E. C. Friedberg and P. C. Hanawalt), pp. 379–401. Marcel Dekker, New York.
Kohn, K. W., C. A. Friedman, R. A. G. Ewig and Z. M. Igbal (1974) *Biochemistry* **13**, 4134–4139.
Magana-Schwenke, N. and B. Ekert (1978) *Mutat. Res.* **51**, 11–19.
Morowitz, H. J. (1980) *Science* **111**, 229–230.
Moule, Y., F. Renauld, N. Darracq and C. Douce (1982) *Carcinogenesis* **3**, 211–214.
Murray, D. and R. E. Meyn (1983) *Br. J. Cancer* **47**, 195–203.
Okada, M., J. Vergne and J. Brahms (1978) *Nucleic Acids Res.* **5**, 1845–1862.
Olinski, R., R. C. Briggs, L. S. Hnilica, J. Stein and G. Stein (1981) *Chem. Biol. Interact.* **34**, 173–183.
Parrish, J. A. (1983) In *Biochemistry and Physiology of the Skin* (Edited by L. A. Goldsmith), pp. 713–733. Oxford University Press.
Peak, M. J. and J. G. Peak (1982) In *NATO Advanced Research Institute Monograph Biological Effects of Solar UV Radiations* (Edited by Calkins), pp. 325–336. Plenum Press.
Peak, M. J. and J. G. Peak (1982) *Photochem. Photobiol.* **35**, 675–680.
Peak, M. J. and J. G. Peak (1982) *Photochem. Photobiol.* **36**, 103–105.
Peak, M. J. and J. G. Peak (1983) *Physiol. Plant.* **58**, 367–372.
Peak, J. G., M. J. Peak and M. MacCoss (1984a) *Photochem. Photobiol.* **39**, 713–716.
Peak, M. J., J. G. Peak, M. P. Moehring and R. W. Webb (1984b) *Photochem. Photobiol.* 613–620.
Peak, M. H., J. G. Peak and L. Nerad (1983) *Photochem. Photobiol.* **37**, 169–172.
Pruckner, F. and A. Stern (1936) *Z. Physik. Chem.* **A177**, 387–397.
Rayshell, M., J. Ross and H. Werbin (1983) *Carcinogenesis* **4**, 501–507.
Rosenstein, B. S. and J. M. Ducore (1983) *Photochem.*

Photobiol. **32**, 361–366.
Rothman, R. H. and R. B. Setlow (1979) *Photochem. Photobiol.* **29**, 57–61.
Schimmel, P. R., G. P. Budzik, S. S. Lam and H. J. Shoemaker (1976) In *Aging, Carcinogenesis, and Radiation Biology* (Edited by K. C. Smith), pp. 123–148. Plenum Press, New York.
Schulein, M., W. N. Burnette and J. T. August (1978) *J. Virol.* **26**, 54–60.
Setlow, R. B. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3363–3366.
Shooter, K. V. and M. Wiessler (1976) *Chem. Biol. Interact.* **14**, 1–11.
Smith, K. C. (1975) In *Aging, Carcinogenesis, and Radiation Biology* (Edited by K. C. Smith), pp. 67–81. Plenum Press, New York.
Smith, P. J. and M. C. Paterson (1982) *Photochem. Photobiol.* **36**, 333–343.
Suzuki, F., A. Han, G. R. Lankas, H. Utsumi and M. M. Elkind (1981) *Cancer Res.* **41**, 4916–4924.
Thomas, J. O. (1975) In *Aging, Carcinogenesis, and Radiation Biology* (Edited by K. C. Smith), pp. 193–205. Plenum Press, New York.
Todd, P. and A. Han (1975) In *Aging, Carcinogenesis, and Radiation Biology* (Edited by K. C. Smith), pp. 83–104. Plenum Press, New York.
Varghese, A. J. and S. Y. Wang (1967) *Science* **160**, 186–187.
Webb, R. B. (1977) In *Photochem. Photobiol. Rev.* (Edited by K. C. Smith), Vol. 12, pp. 169–261. Plenum Press, New York.
Webb, R. B. and M. J. Peak (1981) *Mutat. Res.* **91**, 177–182.
Yamamoto, O. (1975) In *Aging, Carcinogenesis, and Radiation Biology* (Edited by K. C. Smith), pp. 165–192. Plenum Press, New York.
Zelle, B., R. J. Reynolds, M. J. Kottenhagen, A. Schutte and P. H. M. Lohman (1980) *Mutat. Res.* **73**, 491–507.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.